Grazing Impacts of Diverse Zooplankton Taxa on Thin Layers

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LONG-TERM GOALS

The US Navy needs to know how distributions and abundances of light-scattering and sound-scattering organisms in the ocean vary in space and time, particularly in the vertical dimension. Recent field observations have shown that many biological properties may vary substantially over small (e.g. centimeter) scales, commonly referred to as "thin layers" (e.g. Cowles et al. 1998, 1999, Hanson & Donaghay 1998, Holliday et al. 1998, 1999, Dekshenieks et al. 2001, Alldredge et al. 2002, Rines et al. 2002). Our previous ONR-funded research has allowed us to begin to understand how zooplankton interact with thin layers and how they can take advantage of biomass of prey concentrated in these small-scale features (Avent et al. 1998, Bollens 2000, Bochdansky & Bollens 2004, Clay et al. 2004, Ignoffo et al., 2005). However, there is almost no information regarding how zooplankton can influence the characteristics and persistence of thin layers.

In this project we proposed to address this issue, with two main long-term goals: First, to determine to what extent zooplankton graze and export carbon from thin layers; and second, to determine whether and how zooplankton influence the physical (e.g. optical and acoustical), chemical, and biological characteristics of thin layers with their presence. These goals require determination of rate processes such as feeding activity and excretion, which are very difficult to assess in the field. Thus our research is focused on detailed experimental studies of biological rate processes that contribute to the recycling and expert of material in and around thin layers.

OBJECTIVES

The four primary objectives of our proposed research are:

1) To understand the spatial (vertical) coherence and temporal persistence of phytoplankton thin layers with and without the impact of zooplankton grazing.

2) To understand biological rate processes that influence the carbon budget within and in the immediate vicinity of thin layers.

3) To separate local (within thin layers) from non-local (elsewhere in the water column) effects on the thin layers, depending on the type of zooplankton grazers that utilize thin layer organisms as food sources.

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4) To understand to what extent zooplankton return inorganic nutrients to the autotrophs in the layers and thereby influence the persistence and spatial expanse of thin layers.

Our primary hypothesis is that trophic processes alter the flux characteristics of organic carbon and inorganic nutrients such as ammonia and phosphorus in the thin layers and in the immediate vicinity of the layers, thereby changing essential properties of the layers (such as persistence, vertical expanse, biological productivity and export flux). Organisms that aggregate and subsequently stay confined within the layers (e.g. microzooplankton such as ciliates, dinoflagellates or rotifers) will have a different effect on recycling and export fluxes than organisms that only transiently visit these layers (e.g. mesozooplankton such as copepods).

APPROACH

All experiments are being conducted using a plankton tower tank system installed in the Bollens laboratory at Washington State University Vancouver (Fig. 1). This tower tank system has been used successfully in several previous studies (Speekmann et al. 2000, Lougee et al. 2002, Clay et al. 2004, Bochdansky & Bollens 2004, Ignoffo et al., 2005), and was slightly modified for the current project by the addition of valves to allow for 5 cm-spaced subsampling of the tanks in and around the thin layer, as well as installing ethanolamine CO_2 traps to prevent $^{14}CO_2$ release into the atmosphere. In addition to the PI (Bollens) and Co-PI's (Rollwagen-Bollens and Bochdansky), two research technicians from Washington State University Vancouver (Rian Hooff and Angela Gibson) have been responsible for the set-up and maintenance of the tower tank system. Mr. Hooff oversees the operation of the tanks and video recording, and Ms. Gibson is responsible for culturing all experimental organisms as well as monitoring radiation safety, in consultation with the Washington State University Radiation Safety Office.

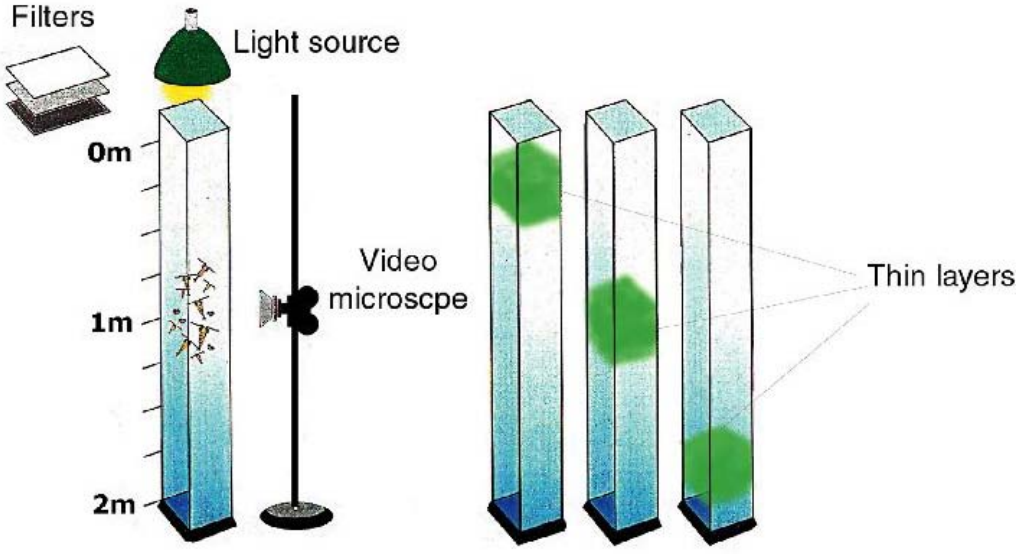

Figure 1. Two-meter high columnar tanks are illuminated by natural light simulators, which incorporate neutral density filters to adjust light intensity. The entire vertical extent of each tank, with a thin layer of phytoplankton, is repeatedly scanned and imaged with an infrared-sensitive video camera to record zooplankton distribution.

Most of the experiments in this research project will be conducted in the first two grant years of the award period (2006 – 2009), which will overlap with portions of the ONR field program: Layered

Organization in the Coastal Ocean (LOCO) being conducted in Monterey Bay, CA. The third grant year will be devoted to data analysis, synthesis and publication. Two graduate students (to be named, one from Washington State University and one from Old Dominium University) will work in tandem on the behavioral and physiological aspects, respectively, of the research.

Effect of microscale distribution of zooplankton: We are determining the fine-scale distribution of phytoplankton in the tanks using an external DFL flourometer as used in Bochdansky & Bollens (2004). Distributions of microzooplankton are determined via direct counts of cells from water obtained through the sampling valves, and mesozooplankton distributions determined via videomicroscopes that regularly pan the length of the tower tank and record onto VHS tapes. Details on the statistical analyses of distributional data resulting from these experiments can be found in our previous thin layer papers (see references above), as well as in Solow et al. (2000) and Beet et al. (2003). For efficiency, these "behavioral" experiments are being conducted simultaneously with the radioisotope experiments described below.

Redistribution of carbon in and around thin layers: We are using homogeneously labeled phytoplankton cells (*Isochrysis galbana*) grown on media containing $NaH^{14}CO_3$ at a concentration of 100 $\mu Ci\ L^{-1}$ (Bochdansky et al. 1999). Exponentially growing phytoplankton cells are gently concentrated with reverse flow filtration, washed with unlabeled GF/F filtered seawater and resuspended to achieve the desired cell concentration for the experiments (Bochdansky et al. 1999). Before introduction into the tower tanks, microzooplankton grazers (*Oxyrrhis marina*) and/or mesozooplankton grazers (*Acartia tonsa*) from laboratory cultures are added to the suspension. As in previous experiments, we also introduce a salinity gradient to the center of the tanks in order to avoid physical mixing of the phytoplankton with the surrounding water (Bochdansky & Bollens 2004). In this fashion, the thin layers in our experiments remain intact for several days.

Carbon ingested by zooplankton is respired, defecated, excreted or incorporated into biomass. Depending on the behavior of zooplankton the carbon in the layers is either recycled within the thin layer or exported. The relative strength of these "active" export fluxes (mediated by zooplankton) to "passive" export fluxes due to cell lysis, diffusion and convective losses of dissolved organic carbon will determine the stability of thin layers in the presence and absence of various types of zooplankton. In the treatments containing zooplankton, grazing will lead to changes in the net fluxes of radioactive carbon, over and above that observed in controls (i.e. without zooplankton).

The redistribution of carbon is monitored over time (48 hours) at 4-12 hour intervals and over the space of the 2 m tower tanks by taking samples through valves positioned along tanks' walls. The samples are size fractionated by filtering through 5 μm, 0.8 μm and 0.2 μm Nuclepore filters so that four pools can be distinguished: a larger and smaller eukaryote fraction (phytoplankton and microzooplankton), the bacterial fraction and dissolved carbon. The dissolved fraction is further divided into an inorganic pool and an organic pool following the procedure in Tamburini & Tedetti (2004). Briefly, a subsample of the filtrate is acidified with HCl and incubated in a polycarbonate flask containing a $^{14}CO_2$ absorbent (i.e., etholamine). The organic dissolved fraction is determined as the difference between the total (sample without acidification) and the inorganic fraction recovered in etholamine. Isotope analysis is being conducted using a Packard Tri-Carb 2100 TR liquid scintillation counter housed at Washington State University Vancouver.

Auxiliary experiments: In order to establish baseline functional responses, we are conducting separate bottle incubations to measure particle removal by zooplankton (*Oxyrrhis marina* and *Acartia tonsa*) grazers. The purpose of these experiments is to establish: i) at what rates prey cells are utilized; and ii) to record diel changes in feeding rates. Most importantly, these experiments will aid in the interpretation of how much grazing itself contributes to the net carbon flux measured with radio-isotopes.

WORK COMPLETED

This project was funded in February 2006, and in July/August 2006 we performed our first set of five time series experiments, in which treatments were interspersed among four tanks. Supplementary functional response experiments established feeding rates of *Acartia* and *Oxyrrhis* on *Isochrysis*, respectively. For the main experiments, the prey concentrations (*Isochrysis*) in the thin layers were chosen to be close to food concentrations that are representative for saturated feeding. The predator levels were selected so that the *Acartia* treatment would fall between the two *Oxyrrhis* treatments in terms of grazing pressure exerted in the *Isochrysis* layer. Samples were retrieved from 11 valves (ten each located on the sides of the tanks and one bottom valve) in intervals of 12 hours and experiments were run for 48 hours.

RESULTS

Figure 2 shows a comparison between initial and final time point for three variables: ^{14}C recovered in the particulate fraction (GF/F filter) and the dissolved organic (after acidification of samples) and dissolved inorganic carbon (by difference between total dissolved and acidified samples). At all grazer levels, phytoplankton in the layer was significantly reduced. In the *Oxyrrhis* treatments, a large portion of the labeled carbon shifted into the lower part of the tank while surprisingly very little ^{14}C was found to settle through fecal pellets in the *Acartia* treatment (Fig. 2).

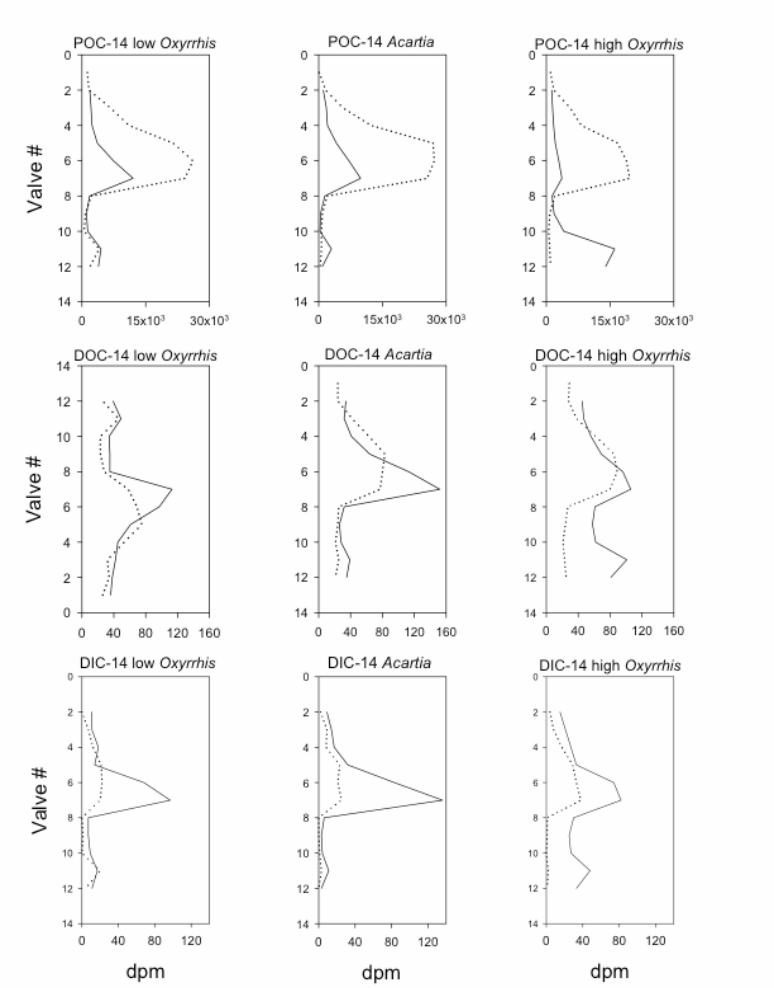

Figure 2. Distribution of carbon-14 in three compartments: Particulate organic carbon (POC), dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) during the initial (dotted lines) and final time points (solid lines, 48 hours). Values are averages of five time series experiments. Note: vertical axis is by valve number, with valve 1 being near the water surface. At all grazer levels, phytoplankton in the layer was significantly reduced. In the Oxyrrhis treatments, a large portion of the labeled carbon shifted into the lower part of the tank while surprisingly very little ^{14}C was found to settle through fecal pellets in the Acartia treatment.

Phytoplankton released significant amounts of dissolved organic compounds and to a lesser extent carbon dioxide into the surrounding water. Predictably, grazers increased the degree of heterotrophy in the system, increasing the amount of inorganic carbon in the water. The ratio of dissolved organic matter production over the release of respired ^{14}C decreased with increasing grazing pressure in the system (Fig. 3). The reason for this initially counterintuitive relationship was that phytoplankton themselves released dissolved organic carbon. By removing phytoplankton biomass, relatively more carbon was channeled into the degradation of organic material and hence into respiration. Normalized to overall grazing pressure, *Acartia* released a lower proportion of DOC to total dissolved carbon than *Oxyrrhis*.

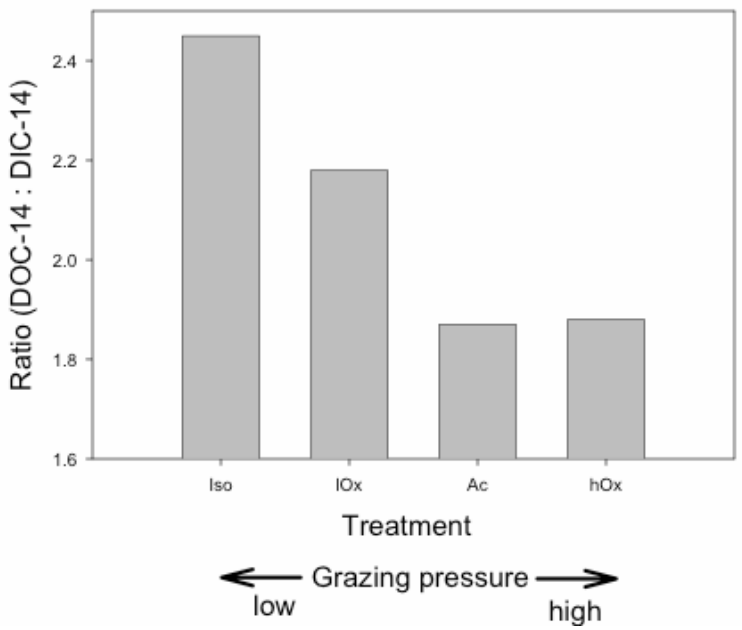

Figure 3. Ratios between integrated dissolved organic (DOC-14) and dissolved inorganic (DIC-14) in four treatments in five time series experiments. Iso = thin layer of Isochrysis without grazers; Ac = Isochrysis with Acartia tonsa, lOx and hOx = Isochrysis with the addition of low and high concentrations of Oxyrrhis marina, respectively. Acartia released a smaller proportion of DOC in relation DIC especially considering that its grazing pressure was closer to the low-Oxyrrhis treatment than the high-Oxyrrhis treatment.

One of the most striking results was the relative amount of $^{14}CO_2$ recovered in traps located above the water column (Fig. 4). The *Acartia* treatment had by far the largest release of carbon dioxide into the atmosphere despite much higher grazing levels in the high *Oxyrrhis* treatments. This was caused by *Acartia* repeatedly moving between the surface of the water column and the thin layer, thereby ingesting radioactive carbon in the thin layers and releasing labeled CO_2 at the surface. *Oxyrrhis*, however, moved deeper into the water column where most cells remained, particularly after the food source in the thin layer was depleted.

Our results demonstrate that microzooplankton and mesozooplankton change the proportion between the release of inorganic and organic carbon due to differences in their physiology. Different behavior between these two groups, however, can drastically influence the direction and the magnitude of carbon fluxes out of thin layers even when data are normalized to the same grazing pressure.

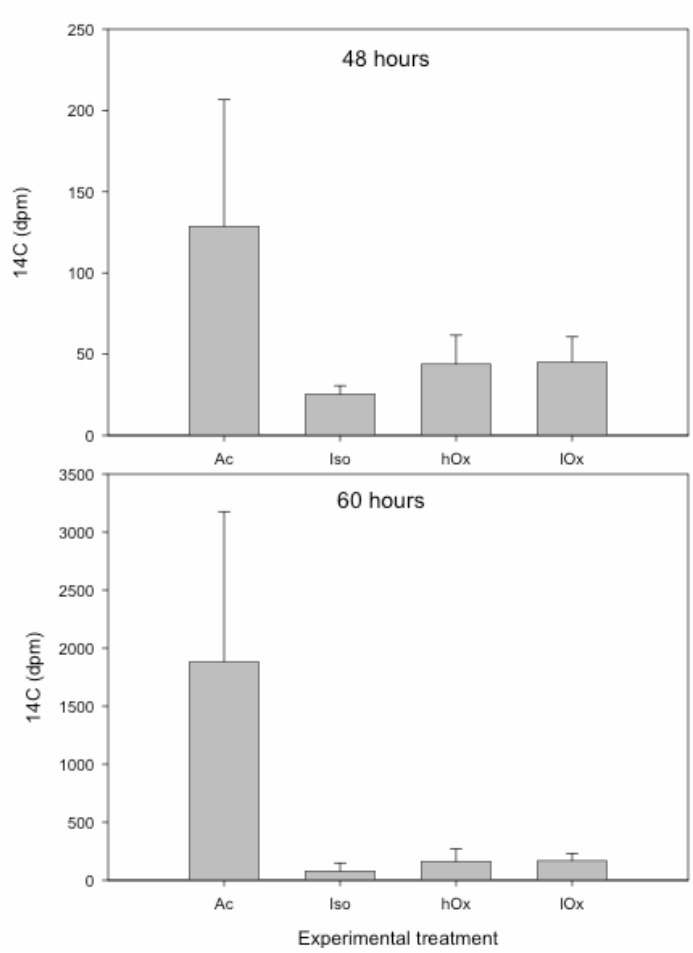

Figure 4. Carbon-14 recovered in the ethanolamine-filled carbon dioxide traps placed above the water columns in the tower tanks. Treatment abbreviations are as in Fig. 3. Acartia tonsa (Ac) released a disproportionate amount of carbon dioxide into the atmosphere due to its vertical migration between thin layer and the surface.

IMPACT/APPLICATIONS

This research will be an important contribution to the Thin Layers program, as it is the first attempt to directly address the influence of zooplankton on rate processes in thin layers. While field studies have to rely primarily on inference from distributions, our controlled laboratory experiments will provide flux patterns of important inorganic and organic nutrients in and around thin layers. In this experimental setting we are able to manipulate predator – prey ratios and available nutrients. We will therefore be able to understand potential effects of zooplankton on the persistence and internal dynamics of thin layers. Our experimental work will provide sufficient data to allow us to make predictions about the contribution of biological processes to thin layer dynamics given the presence and abundance of various zooplankton species found in the field. Our results will demonstrate at which densities micro- and mesozooplankton have the capacity to influence or even control the carbon and nutrient dynamics, and thereby the stability, of thin layers.

RELATED PROJECTS

This research is relevant to virtually all of the many field studies previously and currently being undertaken within the “Thin Layers” program.

REFERENCES

Alldredge, A.L., T.J. Cowles, S. MacIntyre, J.E.B. Rines, P.L. Donaghay, C.F. Greenlaw, D.V. Holliday, M.M. Dekshenieks, J.M. Sulliven and R. Zaneveld. 2002. Occurrence and mechanism of formation of a dramatic thin layer of marine snow in a shallow Pacific fjord. Mar. Ecol. Prog. Ser. 233: 1-12.

Avent, S. R., S. M. Bollens and S. P. Troia. 1998. Diel vertical migration in zooplankton: experimental investigations using video-microscopy and plankton mini-towers. Eos, Transactions, American Geophysical Union 79: 147.

Beet, A., A. R. Solow, and S. M. Bollens. 2003. Comparing vertical plankton profiles with replication. Mar. Ecol. Prog. Ser. 262: 285-287.

Bjørnsen, P. K. and Nielsen, T. G. 1991. Decimeter scale heterogeneity in the plankton during a pycnocline bloom of *Gyrodinium aureolum*. Mar. Ecol. Prog. Ser. 73: 263-267.

Bochdansky, A. B., D. Deibel and R. Rivkin. 1999. Absorption efficiencies and biochemical fractionation of assimilated compounds in the cold water appendicularian *Oikopleura vanhoeffeni*. Limnol. Oceanogr. 44: 415-424.

Bochdansky, A. B. and S.M. Bollens. 2004. Relevant scales in zooplankton ecology: Distribution, feeding and reproduction of the copepod *Acartia hudsonica* in response to thin-layers of the diatom *Skeletonema costatum*. Limnol. Oceangr. 49: 625-636.

Bollens, S. M. 2000. Behavioral Responses of Zooplankton to Thin Layers. ONR FY 2000 Annual Report, Award # N00014-00-1-0173.

Clay, T. W, S. M. Bollens, A. B. Bochdansky, and T. R. Ignoffo. 2004. The Effects of Thin Layers on the Vertical Distribution of Larval Pacific Herring, *Clupea pallasi*. J. Exp. Mar. Biol. Ecol., 305: 171-189.

Cowles, T. J., R. A. Desiderio, M.-E. Carr. 1998. Small-scale planktonic structure: persistence and trophic consequences. Oceanography 11:4-9.

Cowles, T.J., Desiderio, R.J., Twardoshski, M.S., Wingard, C. E., and L. B. Eisner. 1999. Temporal evolution in the composition and structure of small-scale planktonic layers. 1999 ASLO Winter Meeting, Santa Fe, NM, February, 1999.

Dekshenieks, M. M., et al. 2001. Temporal and spatial occurrence of thin phytoplankton layers in relation to physical processes. Mar. Ecol. Prog. Ser. 223: 61-71.

Hanson, A. K.and P. L. Donaghay. 1998. Micro- and fine-scale chemical gradients and layers in stratified coastal waters. Oceanography 11: 10-17.

Holliday, D.V., C. V. Greenlaw, and D. E. McGehee. 1999. Acoustical monitoring of the dynamics of thin layers and aggregations of plankton and fish. 1999 ASLO Winter Meeting, Santa Fe, NM, February, 1999.

Ignoffo, T., S.M. Bollens, and A. Bochdansky. 2005. The effect of thin layers on the vertical distribution of the rotifer *Brachionus plicatilis*. J. Exp. Mar. Biol. Ecol. 316: 167-181.

Lougee, L. A., S. M. Bollens, et al. 2002. The effects of haloclines on the vertical distribution and migration of zooplankton. J. Exp. Mar. Biol. Ecol. 278: 111-134.

Owen, R. W., S. F. Gianesella-Galvão, S. F., and Kutner, M. B. B. 1992. Discrete, subsurface layers of the autotrophic ciliate *Mesodinium rubrum* off Brazil. J. Plankton Res. 14: 97-105.

Rines, J.E.B., et al. 2002. Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. Mar. Ecol. Prog. Ser. 225: 123-137.

Smith, R. E. H., W. G. Harrison and L. Harris. 1985. Phosphorus exchange in marine microplankton communities near Hawaii. Mar. Biol. 86: 75-84.

Solow, A. R., A. Beet, and S. M. Bollens. 2000. Comparing two vertical distributions. Limnol. Oceanogr. 45: 506-509.

Speekmann, C. L., S. M. Bollens and S. Avent. 2000. The effect of ultraviolet radiation on the vertical distribution and mortality of estuarine zooplankton. J. Plankton Res. 12: 2325-2350.

Tamburini, C. and M. Tedetti. 2004. A simple and highly reproducible technique to extract the $^{14}CO_2$ resulting from respiration of 14C-labeled seawater samples. Hydrobiol. 523:1-7.